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Turning a Novel Yeast into a Platform Host for Industrial Production of Fuels and Chemicals

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Cargill

Turning a Novel Yeast into a Platform Host for Industrial Production of Fuels and Chemicals

Metabolic Engineering, June4, 2012

Cargill is an international provider of food, agricultural, risk management, and industrial products and services

- Founded in 1865
- 138,000 employees
- 67 countries
- Over $116 billion revenues, $3.3 billion net earnings in 2009

Nourishing Ideas
Nourishing People

The Role of Biotech at Cargill

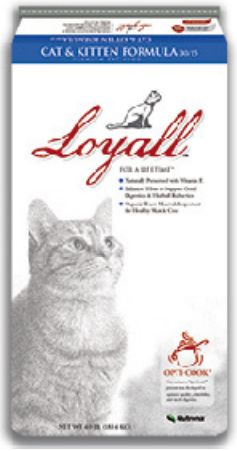
An Enabling Technology that Spans Cargill's Enterprises & Contributes to Top-line Growth and Dollars to the Bottom Line

- ***Create*** New Products and Processes
- ***Enable*** Low Cost Production

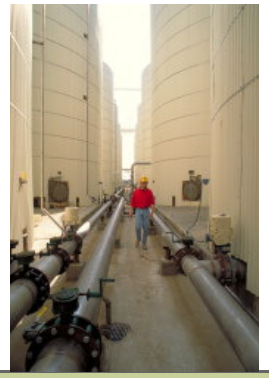
Food

Animal Protein

Feed

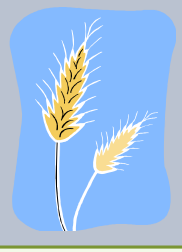


Industrial



BioCatalysis within Cargill:

Feedstocks



Biocatalyst

- Isolated Enzymes
- Whole Cells (yeast, bacteria,

Products

Food :

Citric Acid, Xanthan, Eyrithritol, ARA, HFCS, Probiotics

Feed:

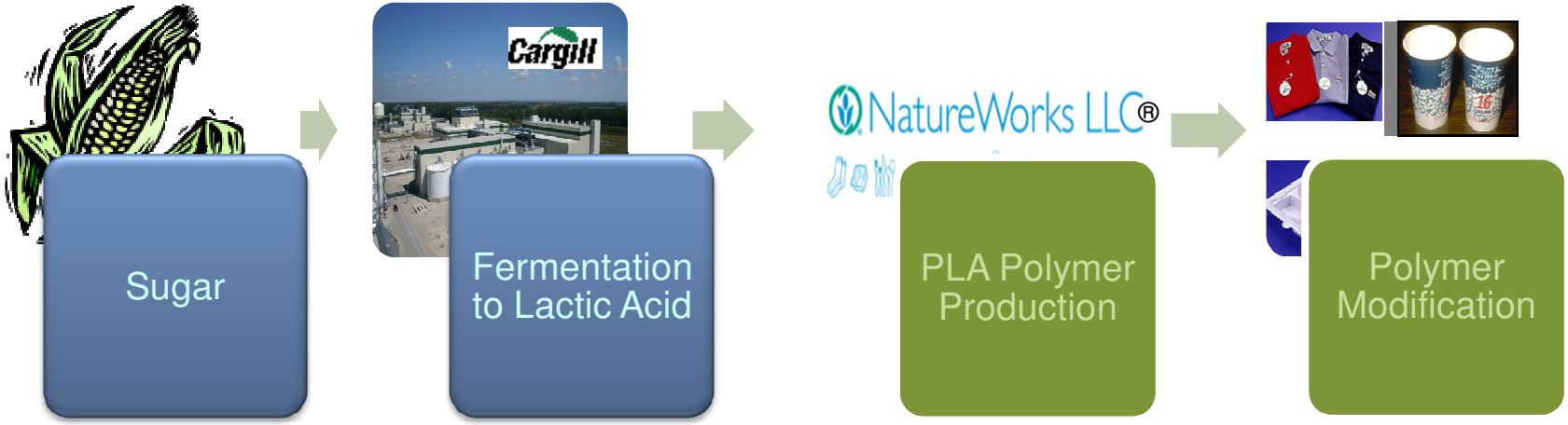
Phytase, Direct Fed Microbial (probiotics)

Cargill Views *Biotechnology* as an *Enabling Technology* that *Contributes to the Bottom Line*

Use Biotech to:

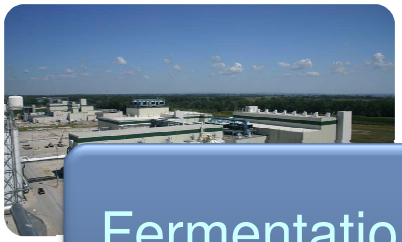
- Create New Processes
- Enable Cost Savings
- Improve Value of Waste Streams

Cargill Is a World Leading Producer of Lactic Acid



- For PLA to compete with petroleum based polymers (PET) lactic acid costs need to be minimized

Traditional Lactic Acid Fermentation



Fermentation to Lactic Acid

Sugar

Bacterial Fermentation

- pH ranges 5.0 to 7.0
- All lactic produced is neutralized; typically with $Ca(OH)_2$

Evaporation

Acidulation

- Free lactic acid from CaLactate salt
- Sulfuric acid is the typical acidulant
- Gypsum produced as waste stream

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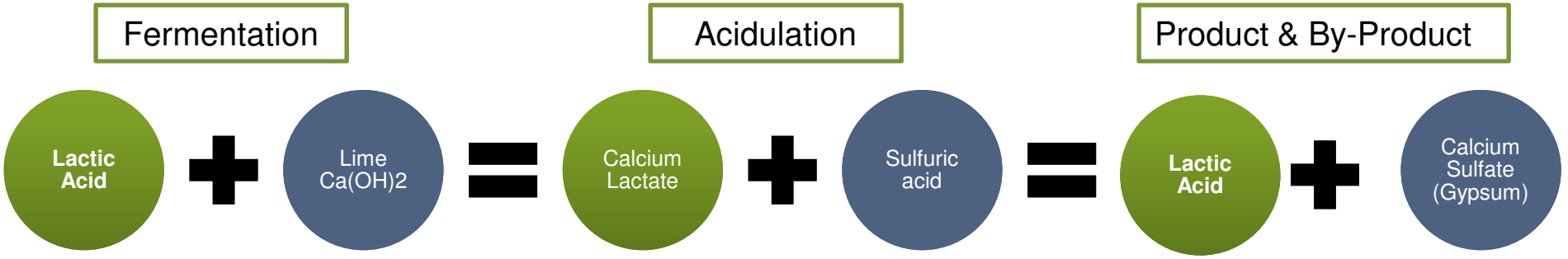
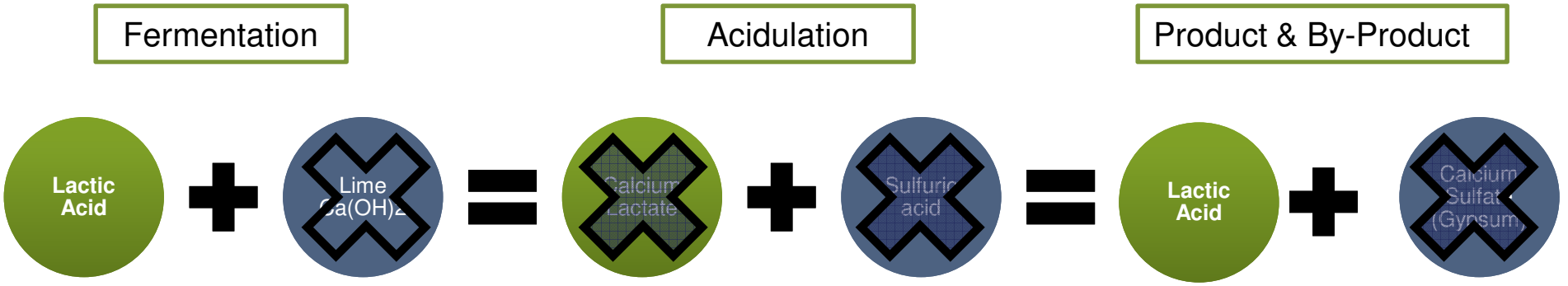
Neutralization and Acidulation – Bacterial process



- Costs: Lime and Sulfuric are major inputs in a neutral pH bacterial process
- Environmental Impacts: Gypsum is a major waste stream

1.2 lbs gypsum made for every 1 lb lactic acid!

Neutralization and Acidulation – Low pH Process



- Operation at low pH can substantially reduce costs and environmental footprint of lactic acid
- Yeast hold the potential to fit this niche
- Additional benefits to yeast for lactic production:
  - Defined media - reduce media and purification costs
  - Increase process robustness - reduced contamination, no bacteriophage

Cargill developed and scaled an efficient industrially relevant low pH yeast for the production of lactic acid.

Challenges of Producing Lactic Acid in Yeast

- Many yeast produce ethanol as the primary fermentation product; blocking this pathway can have serious physiological implications
- Yeasts do not natively produce significant amounts of lactic acid, so must be engineered to do so
  - LDH must be identified
- Lactic acid at pH 3.0 is >85% free, which is inhibitory to most organisms at relatively low concentrations
  - Yeast host strain must be identified
- Lactic acid titers, rates and yields must be competitive with the well established bacterial process

Project decision: Selecting the right host strain

- Do we stick with an established host (e.g. *Saccharomyces cerevisiae*) with established tools?
- Do we consider other hosts?

- (Most metabolic engineering projects at the time stuck with established hosts.)

Choosing a host – a risk/reward decision

Established host

> Large body of historical knowledge

Novel host

> Little to no historical knowledge

We decided to take the risk.

> Know fermentation methods and media

> Shorter development timelines

> Known process robustness

> Known limitations

> Build fermentation from scratch

> Need to build tools before development even starts

> Potential for instability to come out in scale-up

> **Potential to exceed the known limitations of the known strains**

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Novel Yeast (CB1) Identified through Extensive Screening

- Cargill screened ~1200 yeast strains belonging to hundreds of species

- CB1 emerged as a winner and was chosen as a platform biocatalyst

- Key Characteristics of the strain:
  - Tolerant to organic acids at low pH (lactic, acetic, etc.)
  - Fast glycolytic rates
  - Grows and ferments well on a simple defined salts-sugar medium

Consequences of taking the risk:

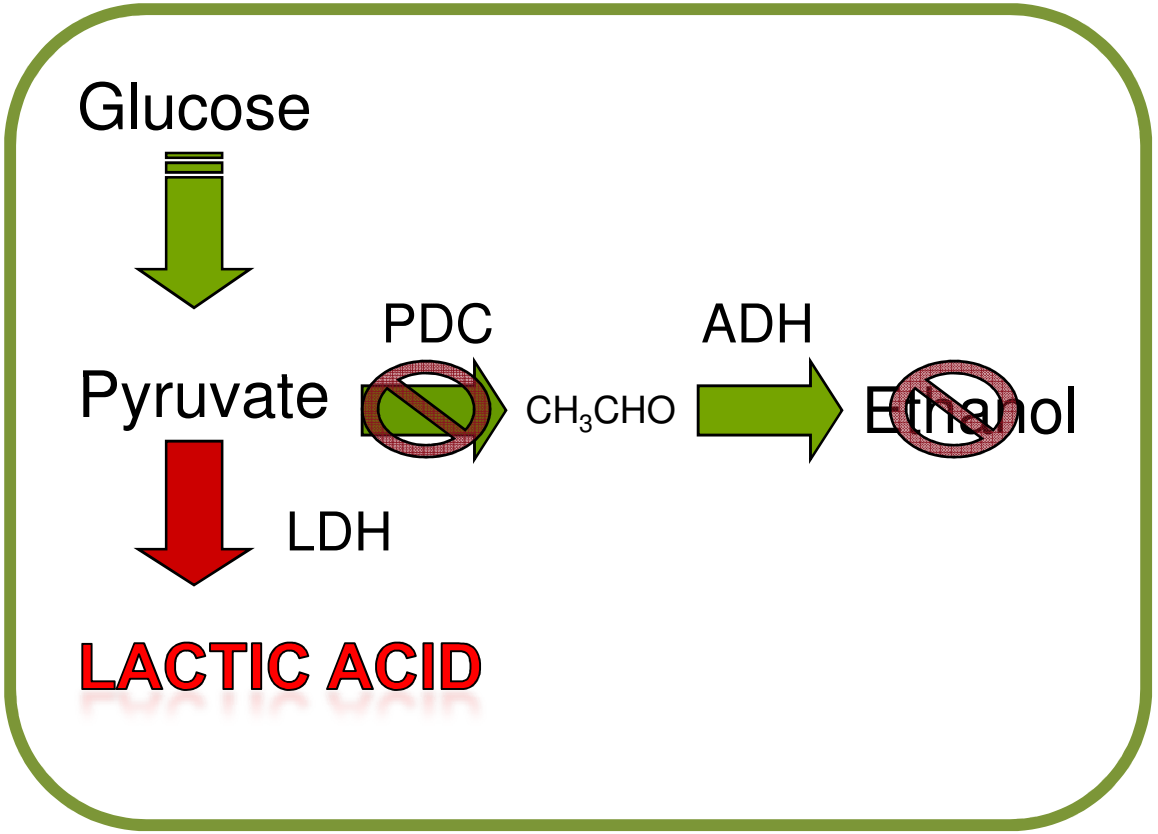
- More than a year of project time was devoted to developing a new host.

- What did we get for our efforts?
  - > A proprietary fermentation host
  - > An effective toolkit for strain engineering
  - > A very inexpensive, salts based, fermentation media
  - > A fermentation process that remains robust at increasing process scale
  - > New limitations that vastly exceed those of the known hosts
  - > A strain with utility that extends well beyond lactic acid

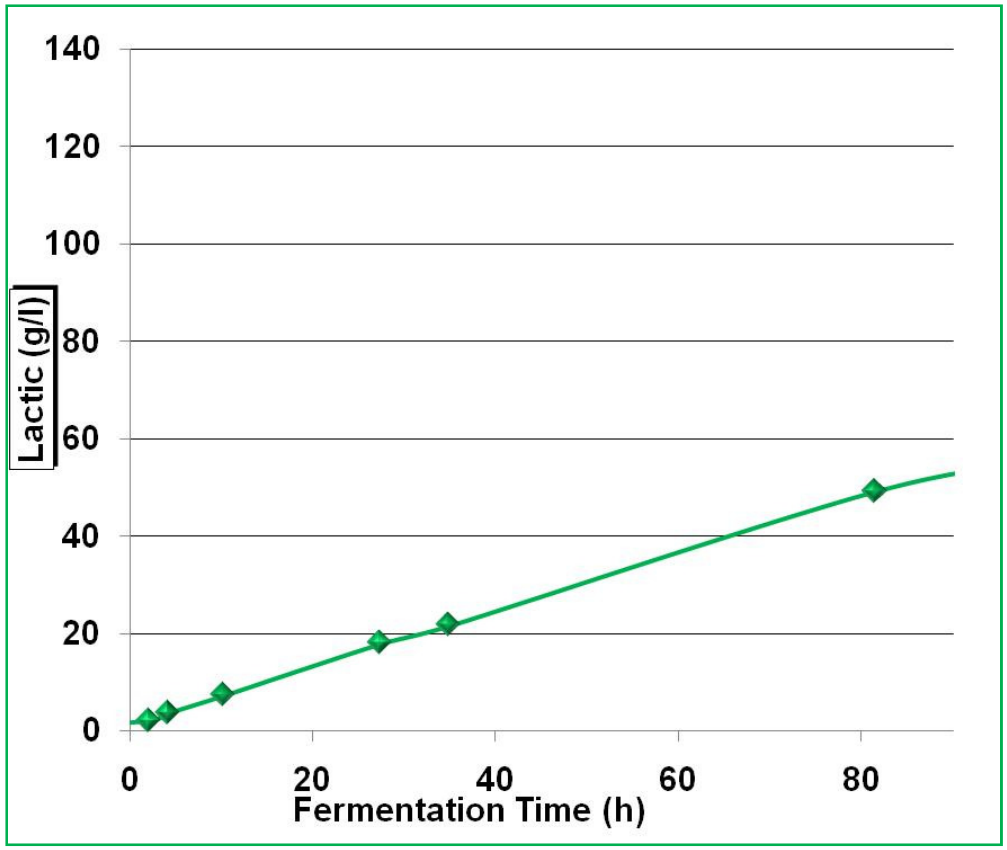
Switching From Ethanol to a Lactic Acid

It's Simple……!

Delete *PDC* gene, Add *LDH* gene



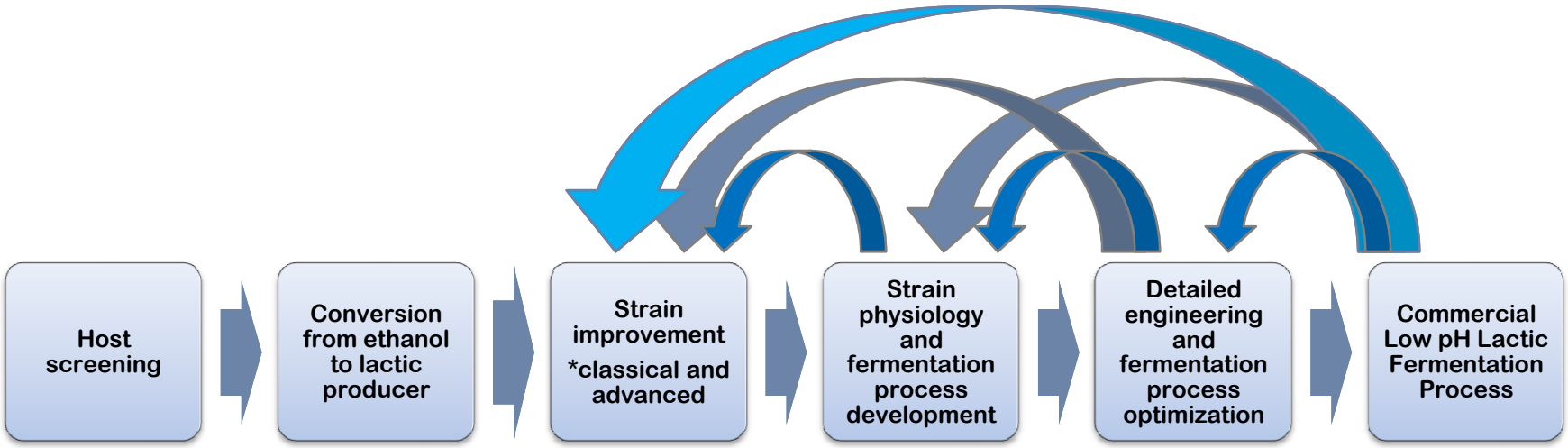
Lactic Acid Production by an Early Generation LDH+ Pdc- Yeast at pH 3.0



However, ….

higher titer, rate and yield needed for commercial process

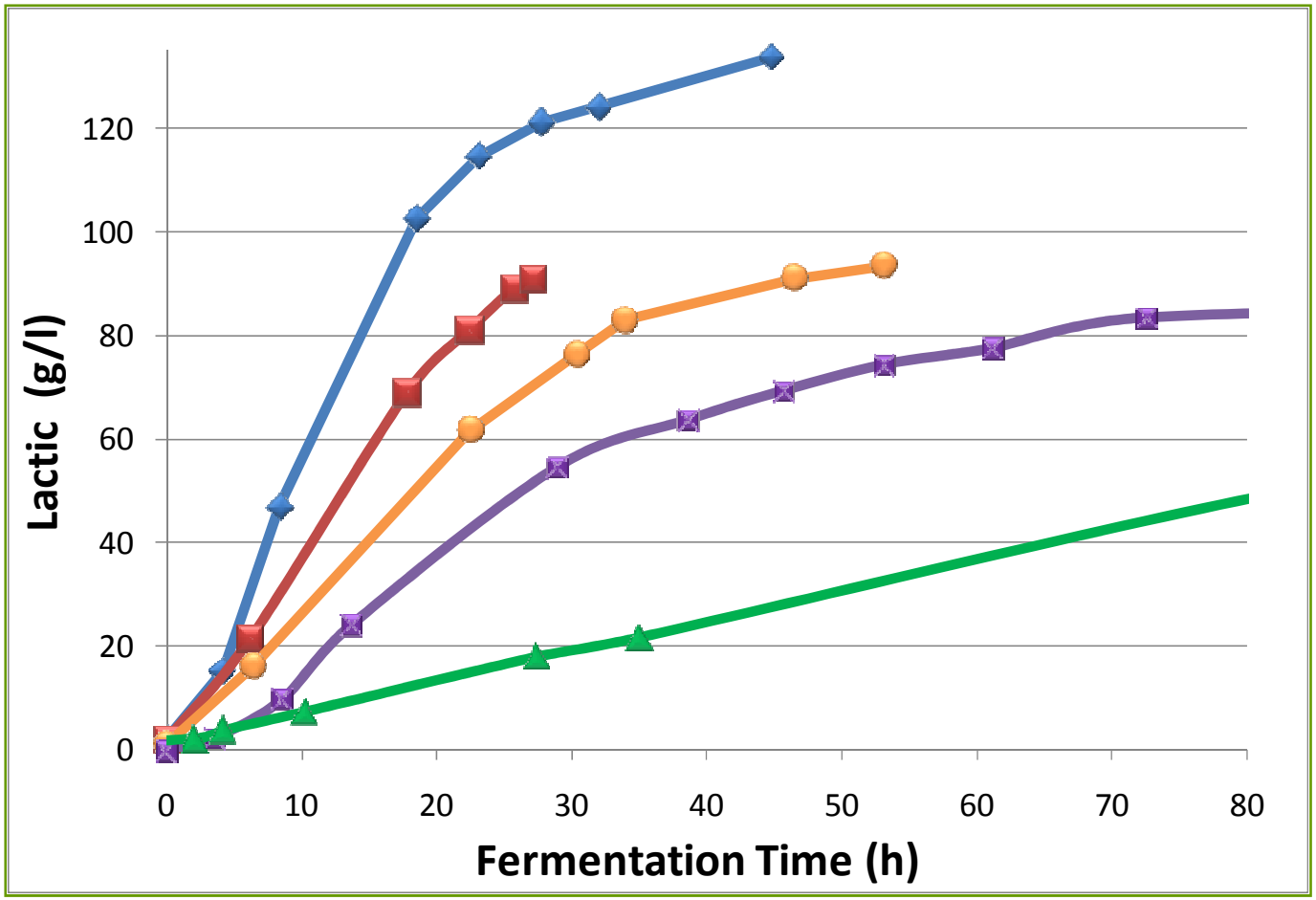
Iterative Approach Used to Improve and Optimize the Strain and Process



Key to making the iterative approach work – Being results driven

1. Robust Strain Benchmarking: Identify successes with confidence. Identify failures early.
2. Persistence: If you can identify failures early, you can fail a lot.
3. Do more of the things that work. Do less of the things that don't.

This Iterative Approach Led to Steady Improvements to the Fermentation at pH 3.0



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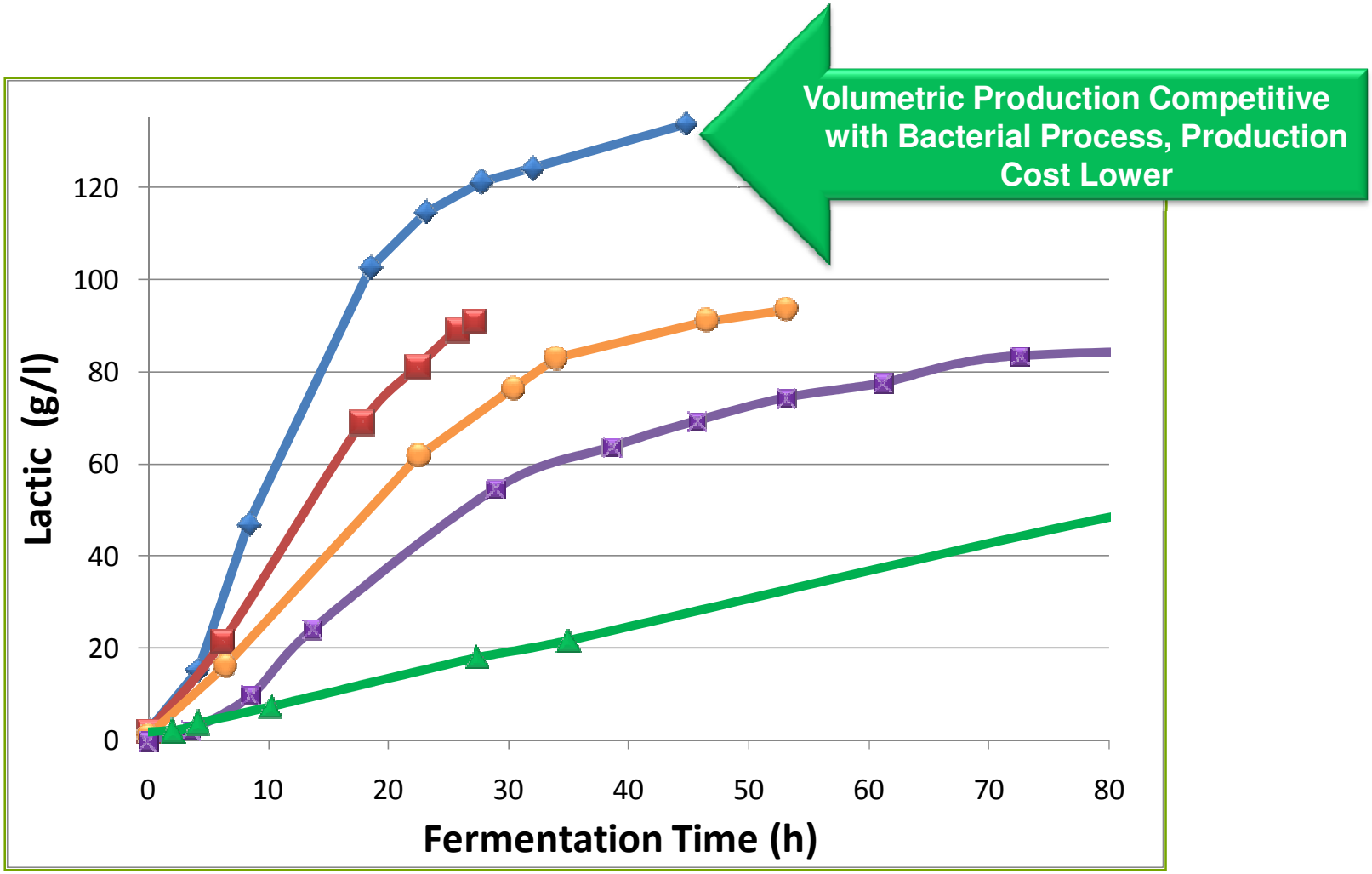
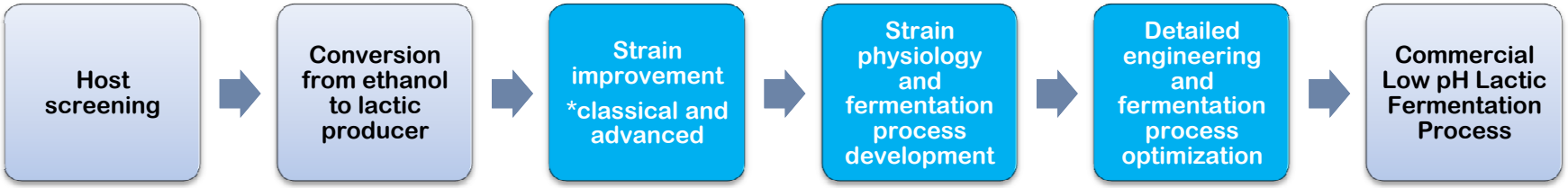
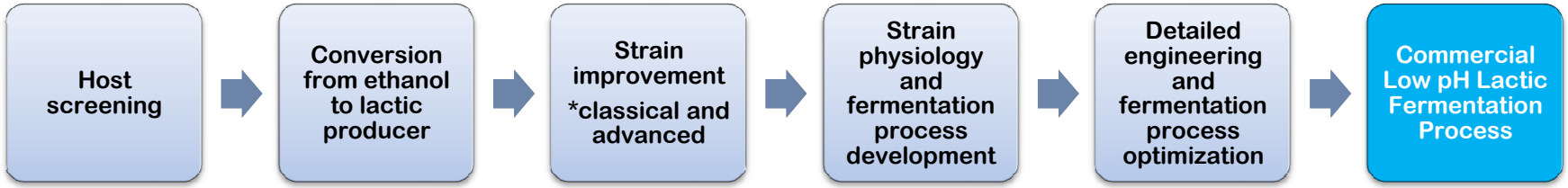


Improvements through Variety of Techniques



- Strain Improvement Tool-Box Developed for CB1
  - CB1 is a non-Saccharomyces yeast so, engineering tools and basic physiology had to be developed
  - Genome was sequenced
    - Faster genetic engineering
    - Pathways identified
    - Gene expression analysis using microarrays
  - Metabolite and flux analysis
  - Chemostat Evolution
  - Classical mutagenesis and selection
- Fermentation Process development and optimization
  - You have to be able to detect progress to make progress.

Successful Commercial Implementation



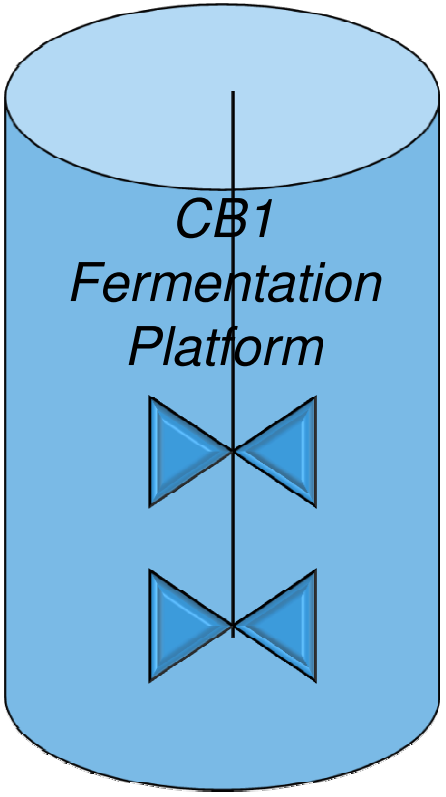
Completed scale-up steps with lactic acid producing yeast:

1. Successful scale-up trials
2. Regulatory Approval
   - MCAN (Microbial Commercial Activity Notice) from EPA in place
3. Bacterial process displaced by yeast process
4. Performance *AND* Economic targets met

Keys to Success – Low pH Lactic

- You get what you screen for in terms of host characteristics – screen for the bug with end objectives in hand
- Diverse tools (genome sequence, arrays, etc.) are key for rapid organism delivery
- Multi-disciplinary approach, with process/engineer involvement as soon as possible;
  - Develop the bug with the process in mind, not process for the bug
  - basic microbiology, classical genetics, metabolic engineering, genome wide tools, analytical chemistry, process development and operations
- Piloting at large scale
  - Increases knowledge base on process sensitivities
  - Increase comfort level of manufacturing and engineering team
  - Early feedback for improved strain development (not all lab and pilot learning translate to scale and visa-versa)

Beyond Lactic Acid: CB1 as a Platform for Fuels and Chemicals



Chemicals:

1) Lactic acid
2) Other chemical building block

Fuels:

1) Ethanol
2) Butanol

Development of CB1 for Cellulosic Ethanol Production

Industrial Challenge of Lignocellulosic biomass Fermentation

- US government (EPA) Renewable Fuels Standard:

Year	Billions of Gallons Cellulosic Biofuel Requirement
2010	0.1
2015	3.0
2020	10.5

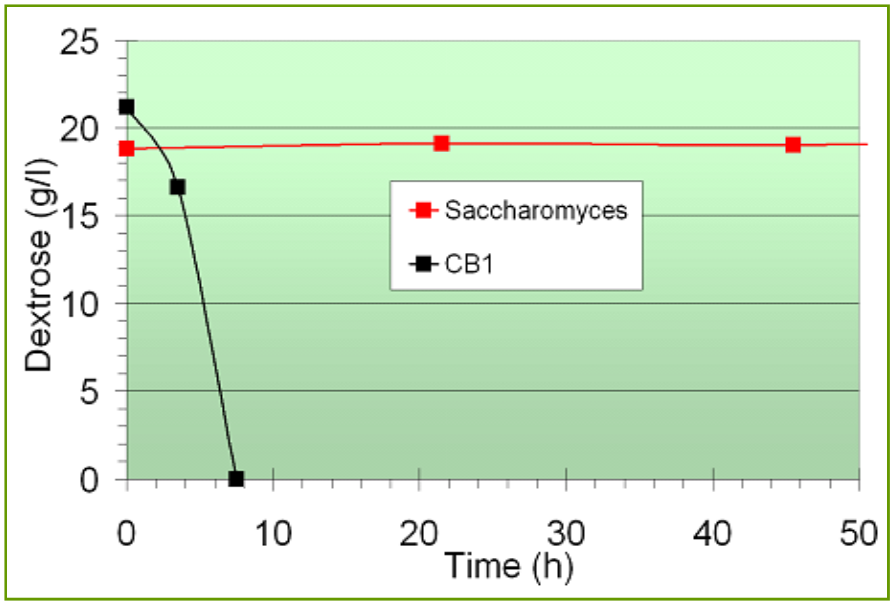
- Cellulosic biomass fermentation to ethanol is key to meeting this standard

- <u>Key Challenges to Cellulosic Fermentation:</u>
  - **Fermentation of hexose *and* pentose sugars** – C5 fermentation is a rare property in yeasts
  - **Inhibitors in feedstock** - acetic acid, furans, phenolics, etc.
  - **Avoid expensive detoxification steps** (e.g. over-liming)

Cargill Has Engineered Our Proprietary Low pH Host (CB1) for Cellulosic Ethanol

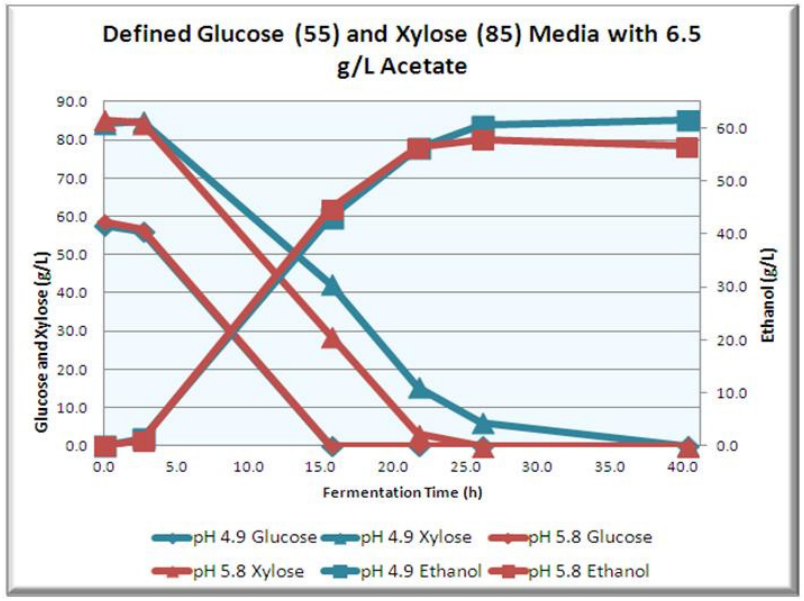
Acetate is a key inhibitor in biomass feedstocks

CB1 has higher tolerance to free acetic acid than Saccharomyces (pH 4.5, 30℃)



glucose only, 10 g/l acetic

CB1 can ferment a typical glucose/xylose mixture in the presence of acetic acid in ~24 hours.



Summary

- **When facing the choice of using a well established host strain or developing a new strain from scratch, Cargill decided to take a risk.**
- **Cargill has developed an innovative yeast biocatalyst and implemented this strain at industrial scale for lactic acid production.**
- **The same host has been engineered for utilization of cellulosic feedstocks.**
- **This biocatalyst is available for licensing for the production of biofuels or chemicals.**

Acknowledgements

Lactic producing yeast:

Strain Development:

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Thank You!

Questions?